REMARKS/ARGUMENTS

Claims 13-17, 22-24, 32, and 35-39 are pending. Claims 1-12 are withdrawn and Claims 18-21, 25-31, 33, 34, and 40-54 are cancelled. Claims 55-96 are new.

The Claims of Group II had been elected for examination. It is our understanding of the restriction requirement dated September 28, 2006 that non-elected Claims 1-12 of Group I and elected Claims 12-17, 22, 23, and 36-39 are directed to related product and process. It is also our understanding that where the Examiner has required restriction between product and process claims and the Applicant has elected claims directed to the product, and the product claims are subsequently found allowable, withdrawn process claims that depend from or otherwise require all the limitations of the allowable product claim will be considered for rejoinder, provided the non-elected process claims include all the limitations of the allowed product claims. In order to preserve the right of rejoinder, non-elected Claims 1-12 have been withdrawn and amended to correspond to the amendments made to the elected claims.

Claims 1, 2, 3, 4, 6, 13-17, 22, and 32-39 have been amended.

Claims 1, 2, 3, 13, and 14 have been amended to recite that the lower eukaryotic host cell is engineered to produce glycoproteins having hybrid or complex N-glycans. Support for the phrase can be found throughout the specification, such as paragraphs [0163-0172] and in particular, the following. Figures 26-28, which show that host cells engineered to produce $GlcNAcMan_5GlcNAc_2$ (strain YSH-1), produce N-glycans having the $GlcNAcMan_5GlcNAc_2$ glycoform. YSH-1 cells are capable of producing the $GlcNAcMan_5GlcNAc_2$ structure because the cells include an *OCH1* deletion, alpha-1,2-mannosidase I, GnT I, and a GlcNAc transporter. The Figures also show that when YSH-1 host cells further include a nucleic acid that expresses GnT III (strain PBP26), they produce N-glycans having a $GlcNAc_2Man_5GlcNAc_2$ glycoform. Figures 29 and 31, which show host cells engineered to produce $GlcNAc_2Man_3GlcNAc_2$ (strains YSH-44 and PBP6-5), produce N-glycans having the $GlcNAc_2Man_3GlcNAc_2$ glycoform. YSH-44 cells are capable of producing the $GlcNAc_2Man_3GlcNAc_2$ structure because the cells include an *OCH1* deletion, alpha-1,2-mannosidase I activity, GnT I activity, mannosidase II activity, GnT II activity, and a GlcNAc transporter. PBP6-5 cells are capable of producing the $GlcNAc_2Man_3GlcNAc_2$ structure because the cells include an *OCH1* deletion, an *Alg3* deletion, alpha-1,2-mannosidase I activity, GnT I activity, GnT II activity, and a GlcNAc transporter. Figures 30 and 32 show that when the host cells further include a nucleic acid that expresses GnT III (strains YSH-57 and PBP38, respectively), they produce N-glycans having a $GlcNAc_3Man_3GlcNAc_2$ glycoform (Figure 30).

Claim 32 has been amended to recite that the lower eukaryotic host cell is engineered to produce glycoproteins having hybrid N-glycans comprising ~~a GnTIII~~ an *N*-acetylglucosaminyltransferase III (GnT III) catalytic activity and a mannosidase II activity. Strain YSH-57 of Example 20 supports the amendment. The YSH-57 host cells are capable of producing hybrid N-glycans because the cells include an *OCH1* deletion, alpha-1,2-mannosidase I activity, GnT I activity, and a GlcNAc transporter. The cells further include GnT II, GnT III, and mannosidase II activities and produce N-glycans having a $GlcNAc_2Man_3GlcNAc_2$ glycoform.

Claims 22 and 36-39 were amended to cancel reference to a cancelled claim.

Claims 6 and 15 were amended to cancel the term "substantially".

Claims 2, 4, 13, 14, 15, 16, 17, 32, 33, 33, 34, and 32 were amended to clarify that the recited activity is "catalytic" activity.

New Claims 55-75 are similar to Claims 13-17, 22-24, 32, 35-39 except the claims are drawn to unicellular and multicellular fungal cells. New Claims 76-96 are similar to Claims 13-17, 22-24, 32, 35-39 except the claims are drawn to yeast cells. Support for new Claims 55-90 can be found in Claims 13-17, 22-24, 32, 35-39 and throughout the specification, for example, paragraph [0147] and [0178], and the Examples, which disclose recombinant yeast hosts that are engineered to make hybrid N-glycans (YSH-1 of Example 13) or complex N-glycans (YSH-44 of Example 15) and which further include a nucleic acid encoding GnT III (Examples 19, 20, and 21).

Paragraph [0001] of the specification has been amended to indicate that U.S. Serial No. 09/892,591 has issued as U.S. Patent No. 7,029,872.

Paragraph [0090] of the specification has been amended to more clearly define what the applicants consider their invention.

I. Claim Objections

Claims 22-24 and 36-39 were objected to for containing non-elected subject matter. The claims objected to are multiple dependent claims that relate back to and depend from both elected claims and non-elected claims. The claims have been amended to remove reference to the non-elected claims.

In light of the amendments to Claims 22-24 and 36-39, reconsideration of the objections is requested.

II. Claim Rejections – 35 U.S.C. § 112.

A. Claims 13-17, 22-24, and 32-39 have been rejected under 35 U.S.C. § 112, first paragraph, as allegedly failing to comply with the written description requirement.

The rejection recites that "[t]he claimed invention is drawn to a lower eukaryotic cell comprising N-acetylglucosaminyltransferase III activity, or additionally having either GnT I, II, or mannosidase activity." The rejection further recites that "[t]he specification discloses that a lower eukaryotic cell encompasses any eukaryotic cell which ordinarily produces high mannose containing N-glycans, including animal, plant, uni and multicellular fungal and algal cells." The rejection then states that "[t]he claimed genus of lower eukaryotic cells thus encompass a large number of cells of different species having very different glycosylation pathways from each other which either naturally comprise the GnTIII and other recited activity or have been genetically modified to express such activity." The rejection further states that "the specification only teaches a method of producing bisected N-glycans in yeast by knocking out genes that are responsible for producing high mannose structures, and subsequently introducing enzymes necessary for formation of complex N-glycan formation, wherein the production of the complex N-glycans in said host cells is the result of concerted action of the combined genetic modification" but "fails to describe other lower eukaryote cells comprising the recited enzymatic activity either naturally or genetically modified, that can produce bisected N-glycan." The rejection concludes that "the specification fails to describe a representative number of species by their complete structure, nor other identifying characteristics" and, "[t]herefore, the specification does not adequately described the claimed genus of lower eukaryotic cells."

The applicants respectfully disagree with the rejection, particularly as applied to the currently amended claims and new claims 55-96. Claims 13 and 14 have been amended to recite a " lower eukaryotic host cell engineered to produce a glycoproteins having hybrid or complex N-glycans " and Claim 32 has been amended to recite a "lower eukaryotic host engineered to produce a glycoproteins having hybrid N-glycans . . .". Thus, the claims are directed to any lower eukaryotic host cell engineered to produce a hybrid or complex N-glycan such as $GlcNAc_2Man_3GlcNAc_2$, $GlcNAcMan_3GlcNAc_2$, $GlcNAcMan_4GlcNAc_2$, or a $GlcNAcMan_5GlcNAc_2$ and further comprising an *N*-acetylglucosaminyltransferase III (GnT III) catalytic activity (Claim 13), *N*-acetylglucosaminyltransferase II (GnT II) catalytic activity and an *N*-acetylglucosaminyltransferase III (GnT III) catalytic activity (Claim 14) or a lower eukaryotic host cell engineered to produce a glycoproteins having hybrid N-glycans comprising an *N*-acetylglucosaminyltransferase III (GnT III) catalytic activity and a mannosidase II activity (Claim 32). New claims 55, 60, and 65 correspond to currently amended claims 13, 14, and 32,

respectively, except that the host cells are unicellular or multicellular fungal cells. New claims 76, 81, and 86 correspond to currently amended claims 13, 14, and 32, respectively, except that the host cells are yeast cells. In addition, the specification has been amended at paragraph [0090] to recite that "[a] lower eukaryotic host cell, when used herein in connection with glycosylation profiles, refers to most typical lower eukaryotic cells, including uni- and multicellular fungal and algal cells."

It is important to understand the similarities and differences between the glycosylation pathway in lower eukaryotes (Figure 1B) compared to the pathway in higher eukaryotes (Figure 1B). In all eukaryotes, the assembly of lipid-linked oligosaccharides in the ER composed of three glucose (Glc_3), nine mannose (Man_9) and two N-acetylglucosamines ($GlcNAc_2$) residues (referred to as $Glc_3Man_9GlcNAc_2$) followed by transfer to a nascent protein and then the removal of the glucose residues and one mannose residue to make a $Man_8GlcNAc_2$ structure is conserved. However, once the protein leaves the ER and enters the Golgi, the glycosylation pathways between lower and higher eukaryotes diverge. Yeast and other fungi typically produce high-mannose type N-glycans by adding up to 100 mannose sugars. In contrast, in most higher eukaryotes, the glycosylation pathway typically involves the removal of mannoses to produce a $Man_3GlcNAc_2$ core and the addition of other sugars such as GlcNAc, galactose, sialic acid, and fucose.

In yeast, the typical glycoprotein is hypermannosylated, which means it has N-glycans having a $Man_{>8}GlcNAc_2$ glycoform. As shown in Figure 1A, an initiating alpha-1,6 mannosyltransferase adds a mannose in an alpha 1,6 linkage to the mannose forming the 1,3 mannose arm of the N-glycan. Once this mannose is added, then hypermannosylation off that added mannose can occur. It has been found that by deleting or inhibiting the initiating alpha-1,6 mannosyltransferase, hypermannosylation is inhibited and the resulting glycoproteins produced in the cell have predominantly $Man_8GlcNAc_2$ N-glycans (*See* Table 1 of the specification). In contrast to yeast, hypermannosylation is not a typical feature in filamentous fungi such as *Aspergillus* (*See* paragraph [0147]).

In higher eukaryotes such as mammals, Man8GlcNAc2 N-glycans are trimmed by an alpha-1,2 mannosidase I to produce $Man_5GlcNAc_2$ N-glycans to which a GnT I transfers a GlcNAc to the mannose on the 1,3 arm of the N-glycan to produce the hybrid N-glycan $GlcNAcMan_5GlcNAc_2$. A mannosidase II then removes the mannoses in alpha-1,3 and -1,6 linkage to the mannose on the 1,6 arm of the N-glycan producing a $GlcNAcMan_3GlcNAc_2$ N-glycan. GnT II then adds a GlcNAc to the mannose on the 1,6 arm of the N-glycan to produce a complex N-glycan having the structure $GlcNAc_2Man_3GlcNAc_2$. The complex N-glycan can

serve as a substrate for various other glycosylation enzymes to produce the N-glycans typically found on glycoproteins produced in higher eukaryotes such as mammals.

Yeast and fungal cells do not contain a mannosidase capable of converting $Man_8GlcNAc_2$ or $Man_{5-12}GlcNAc_2$ N-glycans to a $Man_5GlcNAc_2$ N-glycan that can then be processed to hybrid or complex N-glycans. However, by introducing an alpha-1,2 mannosidase into yeast or fungal cells that do not have initiating alpha-1,6 mannosyltransferase activity, a lower eukaryotic host cell is created that produces glycoproteins that have predominantly $Man_5GlcNAc_2$ N-glycans. The $Man_5GlcNAc_2$ structure is the precursor N-glycan structure that in higher eukaryotes is then modified to make various complex N-glycans. The ability to modify lower eukaryotes to produce $Man_5GlcNAc_2$ structures is key because it is the precursor for all downstream processing steps. In the instant application, the applicants use yeast as a model to illustrate how to make lower eukaryotic host cells that produce glycoproteins having a hybrid N-glycan structure ($GlcNAcMan_5GlcNAc_2$) or a complex N-glycan structure ($GlcNAc_2Man_3GlcNAc_2$, $GlcNAcMan_3GlcNAc_2$, $GlcNAcMan_4GlcNAc_2$).

Lower eukaryotic cells that are deficient in or lack alpha-1,6 mannosyltransferase activity can be obtained, produced, or acquired by any means. *Pichia pastoris*, *K. lactis*, and *Saccharomyces cerevisiae* are but three examples of unicellular fungal host cells which have been genetically engineered to lack alpha-1,6 mannosyltransferase activity or in which mutants have been identified that lack alpha-1,6 mannosyltransferase activity. In yeast, the alpha-1,6 mannosyltransferase activity is encoded by the gene designated as *OCH1* and associated with hypermannosylation of glycoproteins in many lower eukaryotes and yeast that lack alpha-1,6 mannosyltransferase activity will produce glycoproteins wherein the predominant N-glycan has a $Man_8GlcNAc_2$ core structure (Compare Figure 5C with Figure 5B). The application teaches how to identify the *OCH1* gene in unicellular fungal cells (Examples 1 (*Pichia pastoris*) and 9 (*K. lactis*) and how to obtain host cells lacking alpha-1,6 mannosyltransferase activity (Examples 4 and 9). In contrast to yeast, *Aspergillus* and *Trichoderma* are multi-cellular or filamentous fungal species that naturally lack alpha-1,6 mannosyltransferase activity (*See* Maras *et al.* Glycoconjugate J. 16: 99-107 (1999) (Attached as Exhibit A).

Once a lower eukaryotic host cell that is deficient in or lack alpha-1,6 mannosyltransferase activity has been obtained (e.g., yeast or other fungal cell), the host cells can be further modified to include an alpha-1,2 mannosidase activity and a GnT I activity to produce a hybrid $GlcNAcMan_5GlcNAc_2$ N-glycan structure, or further include a mannosidase II activity to produce a complex $GlcNAcMan_3GlcNAc_2$ or $GlcNAcMan_4GlcNAc_2$ N-glycan structure, or further a GnT II to produce a complex $GlcNAc_2Man_3GlcNAc_2$ N-glycan structure.

The applicants teach a library approach that has facilitated identifying suitable catalytic domain/targeting peptide combinations that enable construction of host cells that can make the above hybrid or complex N-glycans. Using the library approach and *Pichia pastoris* and *K. Lactis* as models, the applicants were able to construct host cells that made hybrid or complex N-glycans. Using the library approach, the applicants were then able to construct host cells that included GnT III and were capable of making bisected N-glycans.

In addition, using yeast cells as a model, the applicants exemplified specific embodiments wherein the host cells were deficient in *alg3* activity and alpha-1,6 mannosyltransferase activity. These host cells will produce a $GlcNAcMan_3GlcNAc_2$ when host cells further include an alpha-1,2 mannosidase activity and a GnT I activity and $GlcNAc_2Man_3GlcNAc_2$ when the host cells further include a GnT II activity. As taught above, any one of the above structures can serve as a substrate for a GnT III activity to produce bisected N-glycans.

It is believed that a person skilled in the art and having read the instant application would comprehend and be able to make and/or otherwise identify a variety of other lower eukaryotic host cells lacking alpha-1,6 mannosyltransferase activity using techniques well-known in the art and then using the methods taught in the instant application to genetically engineer any lower eukaryote host cell to be capable of making glycoproteins having a hybrid ($GlcNAcMan_5GlcNAc_2$) or complex N-glycan structure ($GlcNAc_2Man_3GlcNAc_2$, $GlcNAcMan_3GlcNAc_2$, or $GlcNAcMan_4GlcNAc_2$). Finally, such host cells, once made can then be further genetically engineered to express GnT III, and thus produce bisected N-glycans as claimed.

Likewise, a person skilled in the art having read the instant application would comprehend and be able to make and/or otherwise identify a variety of other lower eukaryotic host cells lacking *Alg3* activity. The application teaches how to identify the *Alg3* gene and how to make *Pichia pastoris* and *K. lactis* host cells that lack *Alg3* activity (*See* Example 10). A person skilled in the art can use the information contained within known nucleic acid sequences encoding *Alg3* to scan genomic databases to identify *Alg3* homologues in species for which the *Alg3* gene has not yet been identified. Because the *Alg3* activity is highly conserved across eukaryotes, there is a high probability of success one skilled in the art would be able to identify Alg3 gene in a lower eukaryote and then produce host cells from that lower eukaryote that lack *Alg3* activity. Therefore, it is believed that a person skilled in the art and having read the instant application would comprehend and be able to make and/or otherwise identify a variety of other lower eukaryotic host cells lacking *Alg3* activity using techniques well-known in the art and then

using the methods taught in the instant application to genetically engineer these host cells to be capable of making glycoproteins having a complex N-glycan structure. Such host cells, once made can then be further genetically engineered to express GnT III, and thus produce bisected N-glycans.

Furthermore, it is also well established that the description need only describe in detail what is new or not conventional. See *Hybritech v. Monoclonal Antibodies*, 802 F.2d at 1384, 231 USPQ at 94; *Fonar Corp. v. General Electric Co.*, 107 F.3d at 1549, 41 USPQ2d at 1805. According to the MPEP, "[t]his is equally true whether the claimed invention is directed to a product or process." (MPEP § 2163, section II.A.3(a)). At the original filing date, it was within the skill of the art to isolate or make, test, and identify lower eukaryotic host cells deficient in alpha-1,6 mannosyltransferase activity and/or *Alg3* activity. It was also within the skill of one skilled in the art to follow the teachings in the instant application to introduce nucleic acids encoding various mannosidases and GnTs (including GlcNAc transporters) into a lower eukaryotic host cell having diminished or depleted alpha-1,6 mannosyltransferase activity and/or *Alg3* activity to produce a host cell capable of producing glycoproteins having a hybrid or complex N-glycan structure. Finally, it is within the skill of one skilled in the art to further introduce a nucleic acid encoding GnT III into the above host cells to produce a host cell capable of producing glycoproteins that have bisected N-glycans.

In light of the above, it is believed that the currently amended claims and new claims 55-96 comply with the written description requirement of 5 U.S.C. § 112, first paragraph. Reconsideration of the rejection is requested.

B. Claims 13-17, 22-24, and 32-39 have been rejected under 35 U.S.C. § 112, second paragraph, for allegedly failing to particularly point out and distinctly claim the applicants' invention.

The Examiner has indicated that it was unclear whether the applicants are referring to one of the enzymatic activity (i.e., binding or catalytic activity), the transcriptional activity, or the translational activity. The Examiner suggested that it would be remedial to recite "the acetylglucosamyltransferase III activity."

The applicants have amended claims 13-17, 32-35, and 38 to recite that the activity is "catalytic" activity. Claim 37 has been amended to recite "the" Dol-P-Man:$Man_5GlcNAc_2$-PP-Dol mannosyltransferase activity. It is believed these amendments clarify the metes and bounds of the claims.

The Examiner has indicated that the recitation "the activity is substantially intracellular" renders the claim indefinite because it is unclear whether it means the enzyme is active only inside the cell or both intra- or extracellularly. As the results shown in Figures 33 and 34 illustrate, host cells engineered to express GnT III did not express GnT III in the culture medium.

The applicants have amended Claim 15 to recite that the activity is "intracellular" as it is preferable that the activity exert its effect intracellularly. It is believed the amendment clarifies that the activity is preferably intracellular.

In light of the above amendments, it is believed that Claims 13-17, 22-24, and 32-39 and new claims 55-96 comport with the requirements of 35 U.S.C. § 112, second paragraph. Reconsideration of the rejection is requested.

CONDITIONAL PETITION

Applicant hereby makes a Conditional Petition for any relief available to correct any defect in connection with this filing, or any defect remaining in this application after this filing. The Commissioner is authorized to charge deposit account 13-2755 for the petition fee and any other fee(s) required to effect this Conditional Petition.

Respectfully submitted,

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Enclosure

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